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Significance of *Clostridium difficile* and its cytotoxin in children

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Abstract. Stools of 147 children belonging to different age groups were examined for the presence of *Clostridium difficile*, its cytotoxin and other enteric pathogens. None of the 31 full-term neonates, 5 (16%) of the 32 premature neonates, 27 (46%) of the 59 infants and 1 (4%) of the 25 older children excreted *C. difficile* in their stools. Faecal cytotoxin was only detected in four infants (7%). There was no correlation with diarrhoea, previous antibiotic therapy, type of diet, or the concomitant presence of other intestinal pathogens.

We conclude that colonisation of the intestine by *C. difficile* is probably acquired from environmental sources and that it cannot be regarded as a significant cause of diarrhoea in children.

Key words: *Clostridium difficile* – Antibiotics – Diarrhoea

Introduction

Clostridium difficile is a recognised cause of antibiotic-associated diarrhoea and pseudomembranous colitis in adults [2, 3, 14]. In children however the association of this organism with clinical disease is less clear. Although several paediatric cases of antibiotic-associated diarrhoea [4] and pseudomembranous colitis [15, 18] with *C. difficile* have been reported, the organism is detectable in a high percentage of healthy children below 1 year of age [17].

To establish the prevalence and the pathogenic significance of *C. difficile* and its cytotoxin in children, we have examined the stools of 147 children of different age groups. Stool specimens were also examined for other enteric pathogens. We have also attempted to correlate the isolation of *C. difficile* with the presence of diarrhoea, previous antibiotic therapy, dietary status and duration of hospital stay.

Subjects and methods

1. Subjects. Stool samples were obtained from 147 children hospitalised over a 20-month period at the University of Leuven Department of Paediatrics, Gasthuisberg, Leuven. They were divided into four groups, according to chronological age and/or gestational age at birth.

Group A: *term neonates* (N = 31). All 31 healthy neonates, with a mean age of 4.2 (± 3.7) days, were born at the maternity unit of the University of Leuven Hospital. None of them had diarrhoea.

Group B: *premature neonates* (N = 32). Stool samples from 32 prematurely born infants, with a mean gestational age of 32.2 (± 3.0) weeks, were investigated after an average stay of 24.6 (± 19.4) days in the special-care nursery. Seven of these children had diarrhoea at the moment of stool sampling.

Group C: *infants* (N = 59). These children were further divided into two subgroups. The first subgroup was composed of 27 children with a mean age of 87.8 (± 77.2) days, admitted for acute diarrhoea. Diarrhoea was defined as the passage of unformed or liquid stools with twice the usual daily frequency, causing weight loss and interfering with normal food intake. Other symptoms of intestinal infection often accompanied the diarrhoea: fever, nausea, vomiting, abdominal pain or colics. The second subgroup comprised 32 children without diarrhoea with a mean age of 119.1 (± 113.0) days and admitted for other than intestinal problems.

Group D: *children aged more than 2 years* (N = 25). Three children with diarrhoea, mean age 4.6 (± 2.9) years, and 22 without diarrhoea, mean age 7.2 (± 4.1) years, were investigated.

2. Methods. Bacteriology and parasitology. Stool samples were examined for the presence of *C. difficile* using the selective medium described by George et al. [9], wherein cefoxitin was replaced by cefotaxime 4 mg/l. Plates were incubated at 37°C for 48 h in an anaerobic jar with a gas-generating kit (GASPAK), and examined under a stereomicroscope for colonies with the characteristic morphology. Colonies of *C. difficile* are 4–8 mm in diameter, yellow, with ground glass appearance, circular with a slightly filamentous edge, and flat to umbonate in profile. Typical colonies were further confirmed by the Gram stain.

Stool specimens were also examined for the presence of *Salmonella, Shigella,* enteropathogenic *Escherichia coli* (EPEC), *Yersinia enterocolitica* and *Campylobacter jejuni* using standard techniques. The formalin-ether concentration method was carried out to detect intestinal parasites.

Virology. The cytotoxin of *C. difficile* was demonstrated in monolayer cultures of Vero (African green monkey kidney) cells, maintained in Eagle's minimal essential medium with 8% fetal calf serum. Neutralisation tests using equal volumes of *C. sordellii* antitoxin (Wellcome), diluted 1:50 in phosphate-buffered saline (pH 7.3) were done concurrently. Enterovirus and adenovirus isolation was carried out on monolayer cultures of primary monkey kidney, HeLa, human diploid fibroblast (embryonic skin and muscle) and human rhabdomyosarcoma cells.

Statistical analysis was performed using the chi-square, Fisher exact and Student t-test, as appropriate.

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Abbreviation: EPEC = enteropathogenic *Escherichia coli*

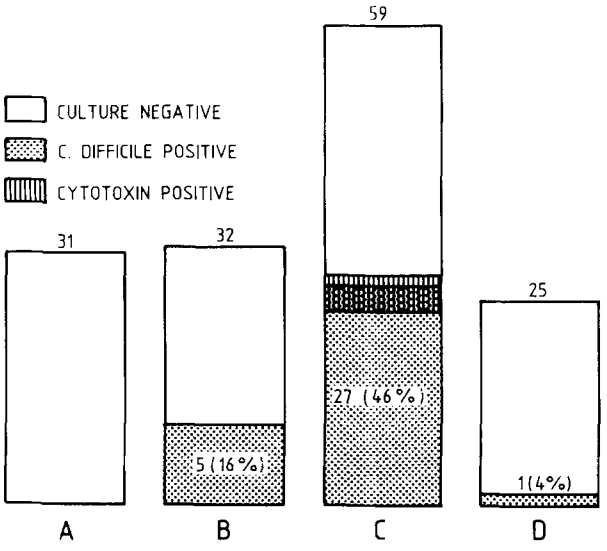

Fig. 1. Number of children with *C. difficile* or/and cytotoxin: A term neonates – B premature neonates – C infants – D older children

Results

The frequency of isolation of *C. difficile* and its toxin from the subjects of the different age groups is shown in Fig. 1. In Table 1 these findings are correlated with the presence of diarrhoea, diet, antibiotic treatment, presence of other intestinal pathogens and duration of hospital stay.

1. Prevalence of C. difficile and its cytotoxin. None of the 31 term neonates (group A), 5 (16%) of the 32 premature neonates (group B), 27 (46%) of the 59 infants (group C) and 1 (4%) of the 25 older children (group D) were found to harbour *C. difficile* in their stools. There was no clustering of cases in space or time. Only four (7%) of the infants had faecal cytotoxin. In one of these four infants, the cytopathic effect, although neutralised with *C. sordellii* antitoxin, was observed in the absence of *C. difficile*. This discrepancy might indicate that the selective isolation medium used was not sufficiently sensitive to detect the organism in all carriers. Of 12 infants with heart disease, 8 were colonised with *C. difficile*, two of them also being cytotoxin-positive.

2. Relationship between C. difficile and diarrhoea. C. difficile was not isolated from the seven prematures nor the three group D children with diarrhoea. Among the 27 group C infants with diarrhoea, 16 (59.3%) were culture positive for *C. difficile*, against 11 (34.4%) among the 32 infants without diarrhoea. The difference between the subgroups is not significant ($P > 0.05$).

3. Diet. Children were breast-fed, formula-fed or treated with parenteral fluids at the moment of sampling. Older children were on normal diets. There were no significant differences in the carrier rates of *C. difficile* between the dietary subgroups.

4. Antibiotic therapy. A respiratory tract infection was the most frequent indication for prior antimicrobial therapy. Most children were treated with a single drug, some a combination of two, most often ampicillin and gentamicin. None could have received antibiotics through breast-feeding. Three (14%) of the 21 prematures, 10 (43.5%) of the 23 infants and none of the older children treated with antibiotics yielded *C. difficile*. The isolation rate was higher, although not significantly so, among infants without antibiotics: 17/36 (47.2%).

5. Enteric pathogens. Salmonella panama was isolated from an infant with gastrointestinal symptoms and a positive culture for *C. difficile*, and from an older child, also symptomatic, but negative for *C. difficile*. An older child with only abdominal pain and a negative culture for *C. difficile*, was positive for *S. heidelberg* and *Campylobacter jejuni*. Two strains of EPEC, respectively belonging to serotypes 055K59 and 011K69, were

Table 1. Recovery of *C. difficile* in different age groups and categories ($N = 146$)

Age groups	A. Term neonates $N = 31$				B. Premature neonates $N = 32$				C. Infants (< 1 y) $N = 59$				D. Older children (> 2 y) $N = 25$			
Recovery of *C. difficile*	positive		negative		positive		negative		positive		negative		positive		negative	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
All children	0	(0)	31	(100)	5	(16)	27	(84)	27	(46)	32	(54)	1	(4)	24	(96)
Toxin positive	0		0		0		0		3		1		0		0	
Diarrhoea present	0		0		0		7		16	(59)	11	(41)	0		3	
Diarrhoea absent	0		31		5		20		11	(34)	21	(66)	1		21	
Breast-fed	0		24		0		7		1		1		0		0	
Formula-fed	0		7		3		8		5		4		0		0	
Parenteral fluid	0		0		2		12		21	(44)	27	(56)	0		0	
Normal diet	0		0		0		0		0		0		1		24	
Previous antibiotics	0		0		3	(14)	18	(86)	10	(43)	13	(57)	0		3	
No previous antibiotics	0		31		2	(18)	9	(82)	17	(47)	19	(53)	1		21	
Stool culture positive for other pathogen	0		0		0		0		1		2		0		2	
Stool positive for virus	0		0		1		2		9		4		0		2	
Duration of hospital stay (in days)			3.7 (±1.6)		40.6 (±22.9)		21.7 (±10.7)		7.8 (±10.7)		4.4 (±6.1)				9.4 (±10.7)	

isolated from two infants without diarrhoea and negative for *C. difficile*.

A poliovirus type 3 strain was isolated from the stool of a 5-month-old infant and probably derived from prior immunosation with oral Sabin vaccine. Echovirus type 33 was discovered in the stool of a premature infant without gastrointestinal symptoms but with a positive *C. difficile* culture. Rotavirus was detected in the stools of two prematures without diarrhoea and in the stool of one older child with diarrhoea, all with negative cultures for *C. difficile*. Rotavirus was also found in seven infants: five yielded *C. difficile* and four experienced diarrhoea.

An adenovirus was excreted by five infants: two had diarrhoea and four harboured *C. difficile*. One older child without diarrhoea had adenovirus but no *C. difficile* in the stool.

6. Duration of hospital stay. At the time of sampling the main duration of hospital stay was 40.6 (± 22.9) days for the prematures with a positive *C. difficile* culture against 21.7 (± 17.7) days for those with a negative culture. This difference is significant ($P < 0.05$).

Discussion

C. difficile was described for the first time in 1935 as a normal component of the faecal flora of healthy neonates [10]. Several reports have confirmed the high carrier rate of *C. difficile* and its cytotoxin in asymptomatic neonates [6, 17], although a Scandinavian survey found toxigenic *C. difficile* in only 2 of 49 children under 1 month old [11].

A recent study of neonates in a special-care nursery showed that 7% of infants aged 1 day, 54% of infants aged from 1–5 days, and 90% of those between 6 and 35 days yielded *C. difficile* on stool culture [1].

Although it is has been speculated that infants may be colonised by the organism during delivery [6], vaginal swabs, collected just before delivery, were uniformly negative for *C. difficile* [1]. This led to the conclusion that infection is mainly from environmental sources, rather than of maternal origin. *C. difficile* has been isolated from environmental swabs in day-care centres [13], in a newborn intensive care unit [12] and also from hands and stools of asymptomatic hospital personnel. After inoculation of the organism onto a floor, it survived for 5 months [12]. In our study, all neonates were born in a recently opened new maternity unit, where the risk of infection from environmental sources may be considered minimal. That might explain why none of the term neonates excreted *C. difficile*.

The colonisation rate was 16% among the prematures and was significantly related to the duration of stay in the hospital. Sheretz and Sarubbi [16] found that the duration of hospitalisation was the factor showing the best correlation with the recovery of *C. difficile* from the stools of premature infants.

Among the infants, 46% harboured *C. difficile* and 7% had the faecal cytotoxin. Similar percentages have also been found by others [7, 11]. In children over 2 years of age the organism could only be recovered in 4%, which is comparable with the 2% prevalence in normal adults found by George et al. [8]. Some investigators however reported a higher prevalence in children with diarrhoea [13] or after antimicrobial therapy [17].

Like others we found no correlation between *C. difficile* and diarrhoea or antibiotic usage [5, 11].

Although breast feeding has been associated with a higher frequency of toxin detection [6], others have found a lower rate of colonisation and no toxin in breast-fed infants [5]. We could not demonstrate a significant difference in the recovery of *C. difficile*, nor in the prevalence of diarrhoea, among children with different feeding regimens.

Our data are too limited to show any relationship between the occurrence of other enteric pathogens and the isolation of *C. difficile*.

We conclude that *C. difficile* should not be regarded as a significant cause of acute diarrhoea in children, irrespective of previous antibiotic usage. Routine stool culture for *C. difficile* in diarrhoeic infants should not be encouraged and could even mislead the clinician. Culture for this organism and a test for faecal toxin should be reserved for those children whose clinical condition is suggestive of pseudomembranous enterocolitis associated with the usage of antibiotics.

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